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1 Assessing the potential of environmental DNA metabarcoding for monitoring Neotropical
2 mammals: a case study in the Amazon and Atlantic Forest, Brazil

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4 Naiara Guimarães Sales^{1*}, Mariane da Cruz Kaizer¹, Ilaria Coscia¹, Joseph C. Perkins¹,
5 Andrew Highlands¹, Jean P. Boubli¹, William E. Magnusson², Maria Nazareth Ferreira da
6 Silva³, Chiara Benvenuto¹ and Allan D. McDevitt^{1*}

7

8 ¹Environment and Ecosystem Research Centre, School of Science, Engineering and
9 Environment, University of Salford, Salford, UK

10 ²Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus,
11 Amazonas, Brazil

12 ³Coleção de Mamíferos, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas,
13 Brazil

14

15 Corresponding authors:

16 Naiara Guimarães Sales (naiarasl@gmail.com)

17 Allan D. McDevitt (a.mcdevitt@salford.ac.uk)

18 Environment and Ecosystem Research Centre, School of Science, Engineering and
19 Environment, University of Salford, Salford, UK

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25 **Abstract**

26 The application of environmental DNA (eDNA) metabarcoding as a biomonitoring tool has
27 greatly increased in the last decade. However, most studies have focused on aquatic macro-
28 organisms in temperate areas (e.g., fishes). We apply eDNA metabarcoding to detect the
29 mammalian community in two high-biodiversity regions of Brazil, the Amazon and Atlantic
30 Forest. We identified critically endangered and endangered mammalian species in the
31 Atlantic Forest and Amazon respectively and found overlap with species identified via
32 camera trapping in the Atlantic Forest. In light of our results, we highlight the potential and
33 challenges of eDNA monitoring for mammals in these highly biodiverse regions.

34

35 **Keywords:** Camera traps; critically endangered; eDNA; river

36 **Running head:** eDNA monitoring of Neotropical mammals

37 **Word Count:** 2500

38 **Introduction**

39 A quarter of mammal species are endangered according to the IUCN Red List of Threatened
40 Species (IUCN 2019) and there is clearly a need for more effective and rapid methods for
41 long-term biomonitoring to be applied across different biomes and over large spatial and
42 temporal scales (Sales et al. 2019a). In recent years, environmental DNA (eDNA)
43 metabarcoding (the simultaneous identification via next-generation sequencing of multiple
44 taxa using DNA extracted from environmental samples, e.g., water, soil) has delivered on its
45 initial potential and is now revolutionizing how we monitor biodiversity (Deiner et al. 2017).
46 The majority of eDNA metabarcoding applications have focused on monitoring fish and
47 macroinvertebrates, with mammals being targeted in only 8% of vertebrate studies (Tsuji et
48 al. 2019). However, with the development of universal primers for vertebrates and
49 mammals specifically, there has been a recent surge in studies tailored to detect and/or
50 monitor mammalian communities in terrestrial and freshwater environments (e.g., Ushio et
51 al. 2017, Harper et al. 2019, Sales et al. 2019a).

52 Recent mammal-focused eDNA metabarcoding studies in temperate regions in the
53 northern hemisphere have relied on well-studied systems with accompanying long-term or
54 historical survey data to test the efficiency of this novel biomonitoring tool (e.g., Harper et
55 al. 2019, Sales et al. 2019a). However, mammal conservation can be more challenging in
56 biodiversity-rich countries as long-term monitoring systems are still scarce outside of
57 Europe and North America (Proença et al. 2017) and ecological field studies used to plug
58 this gap are often hindered due to difficulties in sampling over wide spatial scales. For
59 effective conservation action, adequate knowledge regarding the biodiversity components
60 present in each area is of paramount importance.

61 Environmental DNA from lentic and lotic systems has been found to be effective in
62 not just monitoring aquatic and semi-aquatic mammals, but also terrestrial species (Harper
63 et al. 2019, Sales et al. 2019a). Here, we explore the application of eDNA metabarcoding for
64 Neotropical mammals by verifying its ability to detect aquatic and terrestrial animals from
65 rivers/streams in the highly biodiverse biomes of the Brazilian Amazon and Atlantic Forest.
66 The Amazon is the largest tropical rainforest on Earth, encompassing at least 10% of the
67 world's biodiversity. The Atlantic Forest, which is currently represented by only 11% of its
68 original cover (Ribeiro et al. 2009), is the second most biodiverse biome in South America
69 (WWF 2018).

70

71 **Methods**

72 In the Amazon, water samples (500mL each, in three replicates) were obtained from six sites
73 within three main areas (A-C; Figs. 1 and S1; Table S1). In the Atlantic Forest, water and
74 sediment samples (500mL of water and 25mL of sediment, in three replicates) were
75 obtained from eight sites located in two valleys of the Caparaó National Park (D-E; Figs. 1
76 and S1; Table S1). Temperature and pH were recorded at each site in the Amazon. Mammal-
77 specific universal primers targeting the mitochondrial 12S rRNA gene were used (Ushio et al.
78 2017). A total of 108 samples (including field, DNA extraction and PCR blanks) were
79 sequenced in two multiplexed runs on an Illumina MiSeq platform using the 2 x 150bp v2
80 chemistry. The workflow was conducted following the protocol described in Sales et al.
81 (2019a) and a more detailed description is included in the Supporting Information.

82 Additional data regarding species' distributions in the Atlantic Forest were obtained
83 through camera-trap surveys. Both valleys in the Caparaó National Park were surveyed with

84 terrestrial and arboreal camera traps (Bushnell Trophy CamTM, USA; see Supporting
85 Information).

86

87 **Results and Discussion**

88 Approximately 1.3 million mammal reads were obtained after all the bioinformatic filtering
89 (Amazon – 833,623 reads; Caparaó – 109,233 reads for water samples and 334,593 for
90 sediment samples). Only reads recovered for wild mammals (919,910 reads) were retained
91 for downstream analyses.

92 Overall, we detected 28 Molecular Operational Taxonomic Units (MOTUs) from
93 terrestrial and aquatic mammals, representing eight orders and 14 families (Table S2).
94 Considering a threshold of >0.97 minimum identity, only 13 MOTUs could be assigned to the
95 species level (Table S2). In the Amazon, six species were recovered, with three currently
96 listed as Endangered by the IUCN's Red List (2019) in different categories: the Endangered
97 Amazon river dolphin (*Inia geoffrensis*), the Vulnerable giant anteater (*Myrmecophaga*
98 *tridactyla*) and the Vulnerable lowland tapir (*Tapirus terrestris*). Three Least Concern species
99 were identified: *Thyroptera discifera* and *Rhynchonycteris naso* in the order Chiroptera and
100 the rodent *Toromys rhipidurus*. Detecting *Toromys* is significant as the genus is not known
101 from the area. However, another congeneric species, *T. grandis*, is known from the Amazon
102 River, not far from our study site (Abreu-Júnior et al. 2018). Only one MOTU was detected
103 for each family (Fig. 1).

104 In Caparaó National Park, nine families were detected using eDNA: five in the west
105 side of the park (D) and nine in the east side (E; Fig. 1 and S2). Of these, only seven could be
106 assigned to species level (Table S2). Here, camera-trap surveys detected 17 species (and
107 additional unidentified small mammal species), encompassing 12 families (Fig. S3; Table S3).

108 Combining the two non-invasive techniques, 15 families were detected overall (Table 1), six
109 of them by both methods, three exclusively by eDNA metabarcoding and six solely by the
110 camera traps.

111 More MOTUs were retrieved for the families detected in the Atlantic Forest,
112 suggesting the occurrence of several species of the same family in this area. For example,
113 three MOTUs were recovered in the east side and two from the west side of the Park for
114 both Didelphidae and Cuniculidae. Camera trapping recorded three species of Didelphidae
115 (*Caluromys philander*, *Didelphis* sp., *Philander frenatus*), in accordance with the eDNA data.
116 Only one species from the Cuniculidae (*Cuniculus paca*) recorded by camera traps is known
117 to occur in the Caparaó and the existence of three MOTUs for this family might be due to
118 intraspecific genetic variability or the possibility of cryptic species (within other groups also;
119 Fig. 1). Cricetidae had three MOTUs in the west side of the Park: although this family was
120 not identified by camera traps, several species are described for the Atlantic Forest,
121 including endemic and recently described species (Gonçalves & Oliveira 2014). Furthermore,
122 the Critically Endangered primate *Brachyteles hypoxanthus* was detected using eDNA,
123 demonstrating the detection of arboreal mammals from water samples (e.g., Harper et al.
124 2019).

125 As a similar sampling effort was applied for both areas in this study, there is a need
126 to consider what factors might explain the difference in the number of MOTUs recovered
127 for each biome, particularly if we assume that mammalian alpha diversity should at least be
128 as high in the Amazonian sampling sites as in the Caparaó forest site (see Costa et al. 2000).
129 For example, all the families detected in the Atlantic forest that were not detected in the
130 Amazonian samples are known to occur in Area B of the Amazon (Mendes Pontes et al.
131 2008). DNA degradation in water is one of the main factors reducing detectability over time

132 and limiting temporal inferences. The sampled black waters in the Amazon have low pH
133 (ranging from 3.85 to 4.27), whereas in the Caparaó the reported values are above 6.5
134 (Rodrigues 2015). Acidic environments show higher eDNA decay and lower persistence rate
135 due to the increased degradation of DNA via chemical hydrolysis (Seymour et al. 2018).
136 Therefore, the eDNA recovered in the low-pH waters of the Amazon might be derived from
137 specimens that had recent contact with the water body. Mammal eDNA recovery depends
138 not only on species presence but also on direct/indirect contact with the water system
139 (Harper et al. 2019). The junction of the Negro and Solimões Rivers (area C) has an
140 enormous volume of water and possibly much time had elapsed since it flowed under the
141 forest canopy, but the other Amazonian streams (area B; Fig. 1) are similar in size to those in
142 the Atlantic Forest. In the Amazon, all species/MOTUs were detected in a single replicate,
143 except for the lowland tapir (detected in four replicates in three different streams). This
144 species is known to defecate more frequently in water than on land (Tobler et al. 2010) so
145 this may explain its higher rates of eDNA detection. In the Atlantic Forest, several
146 MOTUs/species were recovered from multiple replicates/sites (Fig. S2), suggesting longer
147 persistence of eDNA in this environment.

148 There is a clear limitation in terms of available DNA sequences in public databases
149 (e.g., Genbank) to match identified MOTUs to species. This issue has been highlighted in
150 previous Neotropical eDNA studies for other taxonomic groups (Cilleros et al. 2019, Sales et
151 al. 2019b). A 12S reference database exists for 164 Amazonian mammalian species in French
152 Guiana (Kocher et al. 2017) and all Amazonian MOTUs were identified to species level here.
153 However, this was not the case for the Atlantic Forest. This biome hosts more than 300
154 mammalian species (more than 50% of medium/large species considered at least
155 Vulnerable; Souza et al. 2019). Therefore, for eDNA monitoring to be implemented in this

156 biome, there is a clear need to generate reference DNA barcodes of a large proportion of
157 the mammalian species present.

158 Here, we demonstrated the potential of applying a cutting-edge and non-invasive
159 molecular approach for biodiversity assessments of Neotropical mammals (including highly
160 threatened species) and would recommend the use of eDNA metabarcoding alongside other
161 non-invasive surveying methods in these biodiverse regions (Harper et al. 2019; Sales et al.
162 2019a). However, significant challenges remain to implement this method in the Neotropics,
163 from a better understanding of the ecology of eDNA within these variable environments to
164 the current lack of appropriate reference sequences for species determination in these
165 biodiversity-rich and anthropogenically-impacted biomes.

166 **References**

- 167 Abreu-Júnior EFd, Charters JD, Percequillo AR (2018) The giant tree rat, *Toromys grandis*
168 (Wagner, 1845): a new record with range extension and comments on its morphology,
169 biology and conservation. *Mammalia* 82: 400-406.
- 170 Cilleros K, Valentini A, Allard L, Dejean T, Etienne R, Grenouillet G et al. (2019) Unlocking
171 biodiversity and conservation studies in high diversity environments using
172 environmental DNA (eDNA): a test with Guianese freshwater fishes. *Molecular Ecology*
173 *Resources* 19: 27–46.
- 174 Costa LP, Yuri L, Leite R (2000) Biogeography of South American forest mammals: endemism
175 and diversity in the Atlantic Forest. *Biotropica* 32: 872–881.
- 176 Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F et al. (2017)
177 Environmental DNA metabarcoding: transforming how we survey animal and plant
178 communities. *Molecular Ecology* 26: 5872–5895.
- 179 Goncalves PR, Oliveira JA (2014) An integrative appraisal of the diversification in the Atlantic
180 forest genus *Delomys* (Rodentia: Cricetidae: Sigmodontinae) with the description of a
181 new species. *Zootaxa* 3760: 1-38.
- 182 Harper LR, Handley LL, Carpenter AI, Ghazali M, Di Muri C, Macgregor CJ et al. (2019)
183 Environmental DNA (eDNA) metabarcoding of pond water as a tool to survey
184 conservation and management priority mammals. *Biological Conservation* 238: 108225.
- 185 IUCN (2019) The IUCN Red List of Threatened Species. Version 2019-2.
186 <http://www.iucnredlist.org>
- 187 Kocher A, de Thoisy B, Catzeflis F, Huguin M, Valiere S, Zinger L, Banuls A-L, Muriene J
188 (2017) Evaluation of short mitochondrial metabarcodes for the identification of
189 Amazonian mammals. *Methods in Ecology and Evolution* 8: 1276-1283.
- 190 Mendes Pontes AR, Sanaiotti T, Magnusson WE (2008) Mamíferos de médio e grande porte.
191 In ML Oliveira, FB Baccaro, R Braga- Neto, WE Magnusson (Eds.). Reserva Ducke: A
192 Biodiversidade Amazônica Através de uma Grade, pp. 51–61. Instituto Nacional de
193 Pesquisas da Amazônia—INPA, Manaus, Brazil.

194 Proença V, Martin LJ, Pereira HM, Fernandez M, McRae L, Belnap J et al. (2017) Global
195 biodiversity monitoring: From data sources to Essential Biodiversity Variables.
196 *Biological Conservation* 213: 256–263.

197 Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic
198 Forest: How much is left, and how is the remaining forest distributed? Implications for
199 conservation. *Biological Conservation* 142: 1141–1153.

200 Rodrigues WF (2015) Concentrações de metais pesados em sedimentos fluviais de leito
201 como sinalizadores de pressões antrópicas no entorno do Parque Nacional do Caparaó.
202 Dissertation. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. 150p.

203 Sales NG, McKenzie MB, Drake J, Harper LR, Browett SS, Coscia I et al. (2019a) Fishing for
204 mammals: landscape-level monitoring of terrestrial and semi-aquatic communities
205 using eDNA from lotic ecosystems. *bioRxiv* 629758

206 Sales NG, Wangenstein OS, Carvalho DC, Mariani S (2019b) Influence of preservation
207 methods, sample medium and sampling time on eDNA recovery in a neotropical
208 river. *Environmental DNA* 1: 119-130.

209 Seymour M, Durance I, Cosby BJ, Ransom-Jones E, Deiner K, Ormerod SJ et al. (2018) Acidity
210 promotes degradation of multi-species environmental DNA in lotic mesocosms.
211 *Communications Biology* 1: 4.

212 Souza Y, Gonçalves F, Lautenschlager L, Akkawi P, Mendes C, Carvalho MM et al. (2019)
213 ATLANTIC MAMMALS: a dataset of assemblages of medium and large-sized mammals
214 of the Atlantic Forest of South America. *Ecology* doi: 10.1002.ecy.2785.

215 Tobler MW, Janovec JP, Cornejo F (2010) Frugivory and seed dispersal by the lowland tapir
216 *Tapirus terrestris* in the Peruvian Amazon. *Biotropica* 42: 215–222.

217 Tsuji S, Takahara T, Doi H, Shibata N, Yamanaka H (2019) The detection of aquatic
218 macroorganisms using environmental DNA analysis - A review of methods for
219 collection, extraction, and detection. *Environmental DNA* 1: 99-108.

220 Ushio M, Fukuda I, Inoue T, Makoto K, Kishida O, Sato K et al. (2017) Environmental DNA
221 enables detection of terrestrial mammals from forest pond water. *Molecular Ecology*
222 *Resources* 17: 63-75

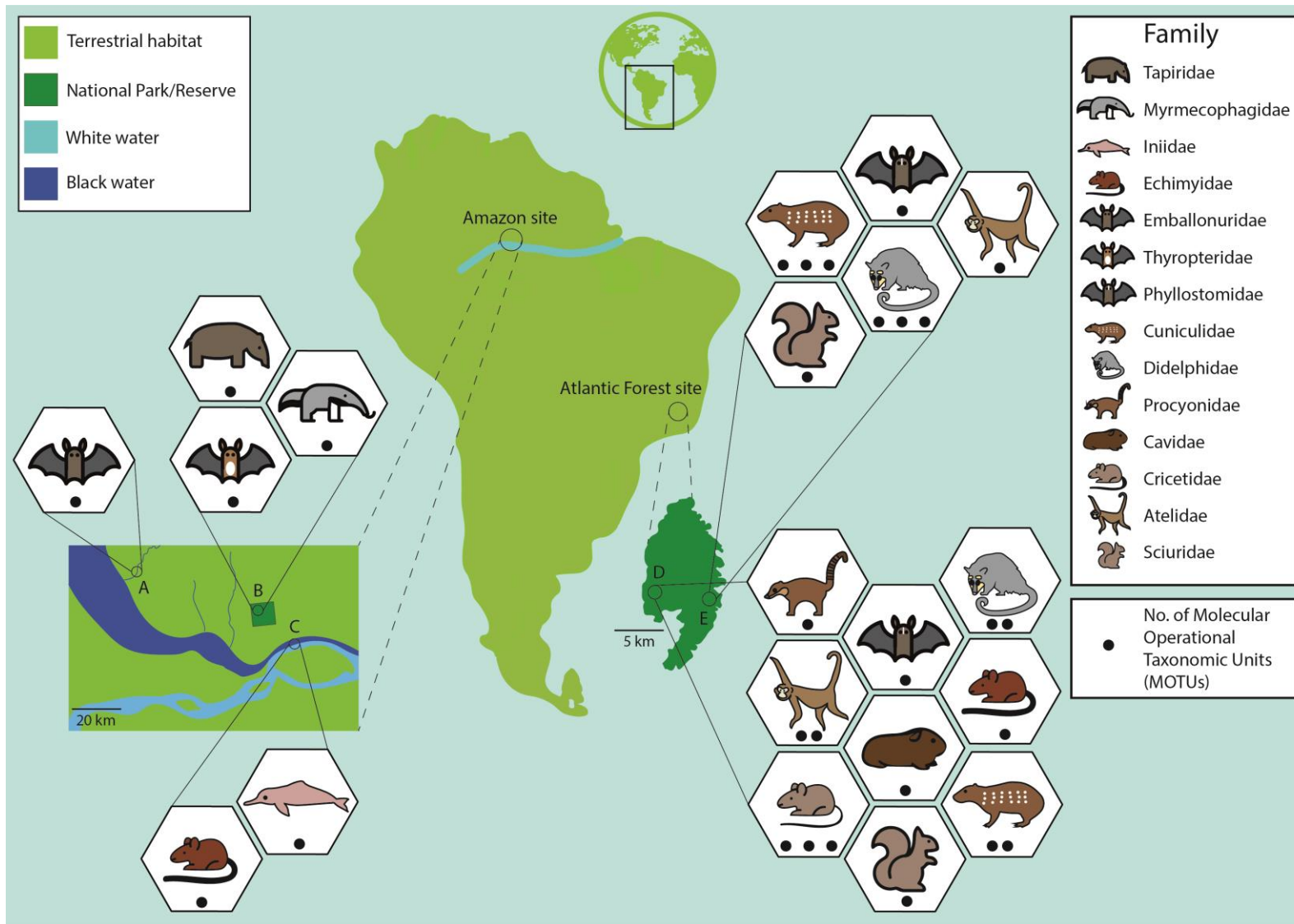
223 WWF (2018) *Living Planet Report - 2018: Aiming Higher*. Grooten M and Almond REA.(Eds).
224 WWF, Gland, Switzerland.

225 **Table 1.** Number (*n*) of species captured with camera traps and number of Molecular
 226 Operational Taxonomic Units (MOTUs) captured with environmental DNA (eDNA)
 227 metabarcoding for orders and families within Caparaó National Park, Atlantic Forest. See
 228 Tables S3 for a more extensive breakdown of camera trap and eDNA data, respectively.

Order	Family	Camera (<i>n</i> species)	eDNA (<i>n</i> MOTUs)
Carnivore	Felidae	1	-
	Mustelidae	1	-
	Procyonidae	2	1
Chiroptera	Phyllostomidae	-	2
Didelphimorphia	Didelphidae	3	3
Pilosa	Myrmecophagidae	1	-
Primates	Atelidae	1	2
	Callithrichidae	1	-
	Cebidae	1	-
Rodentia	Caviidae	1	1
	Cricetidae	-	3
	Cuniculidae	1	3
	Echimyidae	2	1
	Erethizontidae	2	-
	Sciuridae	1	1

230 **Figure legend**

231 Figure 1. Sampling areas for environmental DNA (eDNA) in the Amazon (A-C) and Atlantic
232 Forest (D-E) biomes in Brazil. The families recovered from eDNA metabarcoding in each area
233 are represented by stylized drawings and the number of Molecular Operational Taxonomic
234 Units (MOTUs) recovered within each family is indicated.



235

236 Figure 1.